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AIRBORNE ALLERGENS: ASSESSING
EXPOSURE RISKS*

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THE nature and intensity of exposure to allergens are fundamental determinants of allergic disease. Correlation of these variables with clinical data provides both diagnostic clues and insight into the rational objectives and expectations of therapy. Two components—an indoor (domestic and [often] occupational) one and an outdoor (free air) one—comprise the environment of most people. The first is largely under human control and varies more with social factors and personal taste than geographic location. By contrast, agents derived from natural sources are controlled principally by regional climatic factors and land use. These outdoor allergens defy individual avoidance and control efforts.

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Point-to-point differences in source strength are typical both of indoor and outdoor situations. Because the resulting exposures often differ sharply from regional norms, efforts to document local allergen prevalence remain worthwhile. For this purpose, sampling of airborne particles and field surveys of allergen sources provide complementary data for use by clinicians.

Patterns of particle recovery are especially instructive when source structures are microscopic (e.g., many fungi) or at least not readily apparent. Because many pollens and some important spore types are structurally distinctive, they may be enumerated microscopically in dust deposits. Additional particles, including viable spores of certain imperfect fungi and bacterial cells, while obscure in form, produce recognizable colonies on laboratory substrates. Problems of absolute viability, adequacy of media, and suppression by other growth complicate cultural recoveries. Further, because single colonies may arise from solitary spores or from spore masses, prevalence data are expressed in "colony-forming units." These difficulties notwithstanding, many allergenic aerosols remain for which *no* practical method of enumeration exists; these include animal epidermal materials, defined chemicals, and most arthropod fragments as well as many fungus spores, algae, actinomycetes, and bacteria.

The potential of any technique for monitoring a specific airborne agent depends upon the effective viability, distinctiveness, relative abundance, and aerodynamic size of the particles studied. Particle size affects the collection efficiency of all sampling devices and therefore helps to shape popular concepts of comparative allergen prevalence. Effects of size are shown most clearly by sampling methods requiring fallout of particles on horizontal collecting surfaces such as agar plates or greased microslides. This approach, which until recently was a widely accepted standard procedure (e.g., the Durham sampler), collects particles by fallout and turbulent impaction. Because deposition by both processes varies with the square of particle diameter, it is not surprising that relatively small aerosol types are seriously underrepresented in such gravity collections. Even for large particles, however, the portion of air contributing deposit to a given "catch" is unknown so that data per unit volume cannot be derived. Further, wind speed and direction as well as details of atmospheric turbulence greatly modify deposition, and fallout cannot be assumed to reflect changing levels of allergen prevalence alone. Although these quantitative limitations of gravity slides and open culture plates are widely acknowledged, fallout can provide *trend* data for larger (generally $\geq 20 \mu\text{m.}$) and relatively abundant particles.

However, where studies must define short period changes in prevalence, foster site-to-site comparisons, or monitor the relative prevalence patterns of small or less common agents, other sampling approaches are mandatory.

Two principal methods now provide data in volumetric terms (i.e., as particles/meter³). Suction traps and related devices aspirate air at fixed rates into flow channels with one or more sharp bends. Particles unable to change direction quickly tend to cross stream lines of flow and to strike the channel walls at predictable points where sampling surfaces are positioned. The resulting devices have high efficiency for a wide range of solid aerosols, including small spores. In addition, if jets of decreasing caliber are designed into a device, it will provide deposits, each characterized by a discrete range of aerodynamic sizes (e.g., cascade impactors). Suction traps must be directed continuously into the wind for efficient operation and should maintain intake air speeds equal to those of the ambient atmosphere to minimize collection errors.

A second type of sampler recovers particles impacted by paired, narrow, adhesive-coated surfaces which are whirled rapidly through air. The volume swept out by such a device is readily calculated as the overall product of the total sampling area, the circumference of rotation, revolutions per minute, and minutes of operation. The probability that a particle in the path of a collecting surface will be struck is a function of particle size and, in practice, exceeds 90% for particles the size of ragweed pollen (ca. 20 μm). Collection efficiency falls rapidly for progressively smaller particles but may be estimated, at least, for all spherical aerosol types. Differences in wind direction do not affect recovery, and changes in wind speed apparently have minimal effects below 33 k.p.h. In practice, lucite rods ("rotorods") or 1 mm. microslide edges have provided suitable surfaces.

For particles the size of most pollen allergens, suction traps and rotating impactors provide comparable prevalence estimates; for small spores, recovery by the former group clearly is superior. However, absolute efficiency values are not available for the devices and surface coatings currently in use. Instead, data are best expressed as "recoveries/M³ of air sampled" using a standardized exposure technique including suitable adhesives (e.g., silicone greases).

Awareness of local environment is rewarded by many clues to allergen prevalence. Events, including the swarming of insect species, often are grossly evident and an appreciation of local rainfall, vegetation types, and

cropping practices offers insight into probable levels and types of prevalent airborne fungi. Potential "hay fever plants"—although unidentified—may be suspected by a floral pattern of numerous, grouped, drab, scentless florets. Floral surveys by an informed observer can, in addition, define the current status and anticipated output of major pollen sources in a locality.

The potential value of visits by the allergist or environmentalist to patients' homes and work places deserves emphasis. This approach can quickly define irritant exposures to certain dusts and gases and emphasize point sources of animal dander and of specific pollens. Brief continuous air samples obtained by compact mechanical collectors provide complementary data implicating covert sources of exposure. Systematic observations of furnishings and of soiling help to quantify dust burdens and facilitate application of necessary avoidance measures. Special attention to air modifying equipment is justified, both to achieve its most beneficial effects and to perceive potentially harmful microbial contamination.

Microscopic analysis of air samples, whatever their source, remains the basis of prevailing concepts of exposure to airborne pollens and estimation of time trends and site-to-site differences. Grains are examined when expanded in water-based media containing fuchsin, which stains the outermost layer of the pollen wall (exine). The appearance of grains so prepared best reflects features of the exine although the clear inner wall layer (intine) and cell contents also contribute.

Under ideal circumstances, most airborne pollen types may be identified with a genus or larger grouping of source plants. However, obscuration, aberrant structure, or an unfavorable position may deny this promise for many grains that appropriately remain "unidentified." In addition, members of certain large groups (e.g., the grasses; the goosefoot and amaranth families) produce grains that are practically alike by light microscopic criteria.

Most pollens appear as single, roughly spherical structures with one or more apertures which are elongate (furrows) and/or spherical (pores). Deviations from this pattern, including fused tetrads and polyads, grains with air-filled bladders, elongate and inaperturate grains, often facilitate rapid recognition. Similarly, grains with single pores (grasses and allies) or single furrows (palms, ginkgo, etc.) are identified readily. For that majority of pollens with three or more apertures, discrimination is more exacting because the appearance of a grain varies markedly with its position or attitude. Usually, the centers of apertures are aligned in a specific meridian (the "equator") midway between two aperture-free areas

(the "poles"). Orientation is assisted by first determining whether a grain presents with polar or equatorial aspect uppermost; thereafter, details of the exine surface and apertures may be traced systematically. Both optical and surface sections provide points of differentiation and aid in deriving the three-dimensional concept of grain structure essential for identification. A limited number of illustrated references¹⁻⁴ can assist this process. However, a set of locally collected pollens from authenticated sources will be required for most comprehensive surveys. Where such "knowns" are unavailable, a systematic key, as provided in Table I, can facilitate identification.

Fungal spores that may be encountered on particulate samples include reproductive units of, probably, 40,000 species of fungi adapted for airborne dispersal. These particles range in size from 1 to 300 μm ., and many types fall within the size range 5 to 50 μm . Fungus spores display a wide range of shapes—from spherical to threadlike—although many are elliptical or fusoid; some also bear distinctive spines or appendages. One or two-celled as well as multicellular forms occur, and septa may be transverse only or both transverse and longitudinal. Fungus spores range from colorless through a broad range of yellows, browns, greenish browns to black. Many unrelated forms share apparently identical spore types, and closely related taxa may have extremely diverse spore morphology. In addition, many fungi produce more than one morphologically distinct spore type during their life cycles.

Numbers and kinds of fungus spores in air depend both on local patterns of release and long distance transport. Day-to-day and even hour-to-hour levels tend to be extremely variable. Seasonal effects on prevalence patterns tend to involve qualitative rather than quantitative differences except during winter periods with significant snowfall when airborne spore levels approach zero. Circadian variations are not well studied, although dark spores tend to dominate midday collections and basidiospores often are abundantly released at night. Substrate distribution profoundly affect low elevation (1 to 2 m.) spore patterns while prevalence trends at higher elevations (rooftop and above) are more strongly affected by long distance transport.

Environmental factors that may contribute to these fluctuations include rain, which fosters wash-out of some spore types but release of ascospores and dispersal of yeasts; wind, which increases levels of "dry weather" spores (e.g., *Alternaria* species); and changing relative humidity. Human

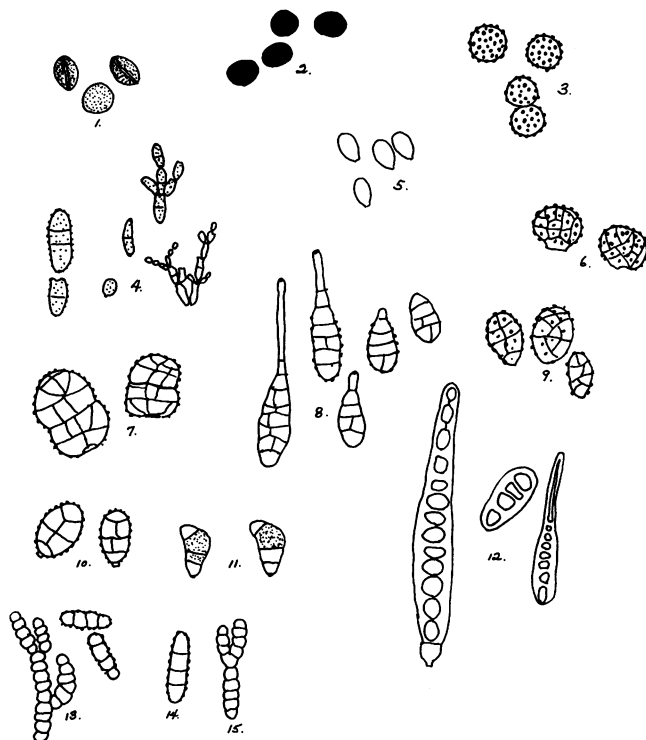


Fig. 1. Airborne spores of common imperfect fungi

activity causes spore release from natural substrates, but the exact effects of most backyard and commercial pursuits remain unstudied.

Although more than half of airborne fungus spore types are colorless, spherical to elliptical structures in the 2 to 6 μm . range that defy identification, many common types are recognizable by spore morphology. The most abundant and widely encountered fungus spores are those of *Cladosporium* species. Other very common dark-spored taxa include *Alternaria* and *Epicoccum*. These and other dark-spored imperfect fungi (viz., with spores produced asexually) tend to predominate during dry weather and are relatively readily identified. Sexually produced spores that are common in air include ascospores (cup fungi) and basidiospores (derived from mushrooms, rusts, smuts). Ascospores are especially frequent after rain and may outnumber all other spore types at that time.

Most ascospores are not currently identifiable to genus; however, those of *Leptosphaeria* species are recognizable and appear especially common.

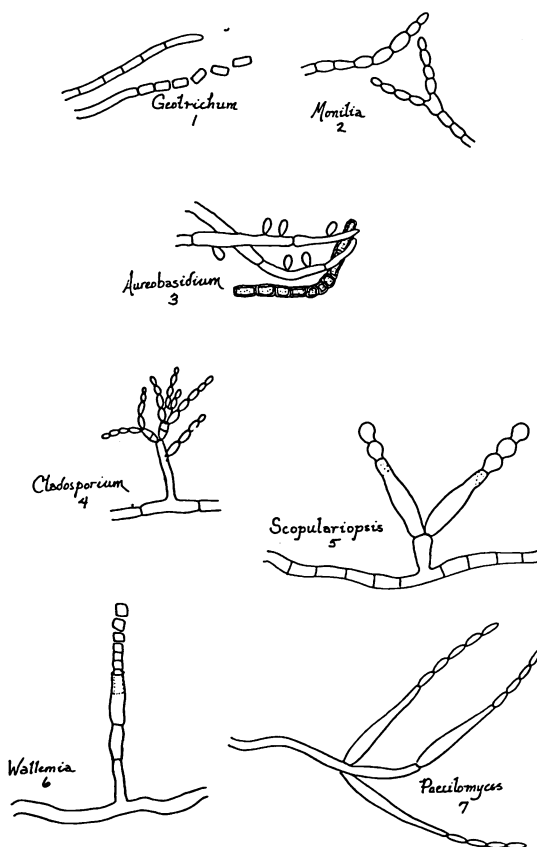


Fig. 2. Fungi commonly recovered in culture

Basidiospores are second in abundance only to *Cladosporium*; again, most are not readily identifiable to genus. Common types that can be recognized with some confidence include *Ganoderma* and *Coprinus*, spores of rusts, and smuts. Spores produced by other plant pathogenic fungi such as the downy and powdery mildews also are often recognizable by light microscopy. However, at present the allergist's greatest interest is properly directed to a group of airborne, pigmented, asexual fungus spores. Table II provides a key to the sources of these common, dark spores, based on spore morphology, which is depicted in Figure 1.

Cultural techniques are often helpful in the enumeration of spore types that lack distinctive form, including species of *Aspergillus* and *Penicillium*. Culture samples underestimate spore prevalence by a factor that increases with the absolute levels present. Using colony counts, calculated

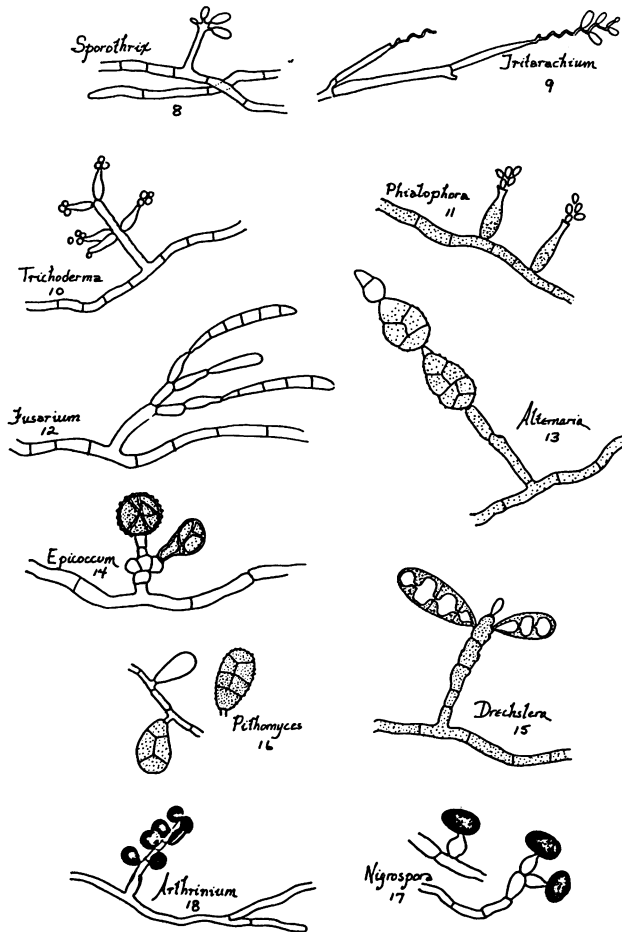


Fig. 2. (continued)

recovery efficiency varies from 0 to 75%, depending on spore types and sampling conditions. Many spores will not grow on artificial media, distorting the qualitative picture, and, of course, only viable spores are recovered. Various media can stimulate or inhibit growth rate as well as sporulation and affect colony morphology; some media selectively inhibit or stimulate specific taxa. Malt extract agar is a useful general purpose medium for the fungi. Optimum temperature for culturing most fungi is near room temperature. A few taxa are thermotolerant and grow over a wide range of temperature (e.g., *A. fumigatus*) while others require high temperatures (45 to 55° C.) for optimum growth. Many fungus colonies thrive at room temperature but continue to grow slowly at 4° C. These temperature characteristics can be manipulated selectively to isolate par-

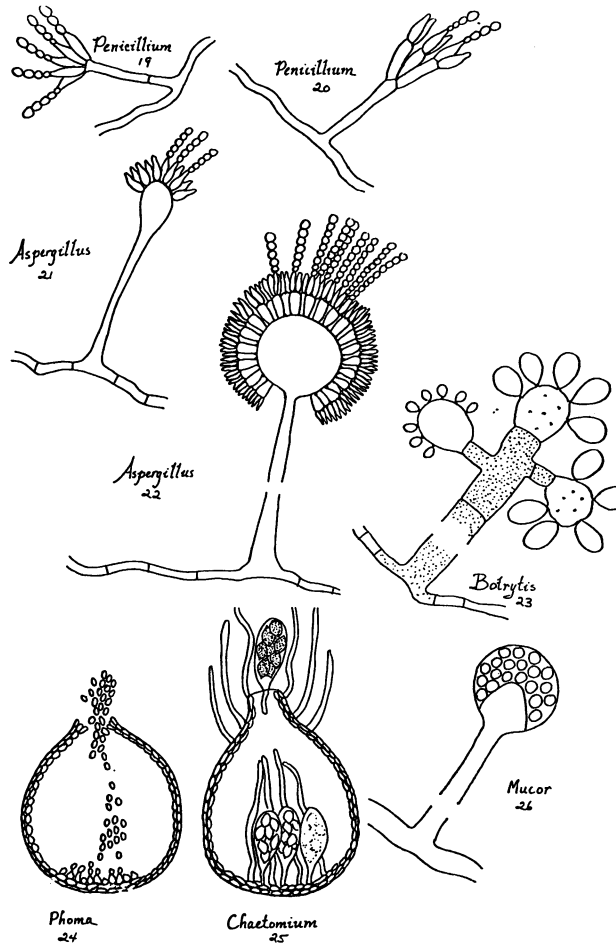


Fig. 2. (continued)

ticular fungi. The vast majority of fungi—and all those of interest to allergists—are aerobic.

Identification of fungi in culture requires that spores be present and that their mode of production is discernible. Spore characteristics mentioned above as well as characteristics of vegetative growth, spore-bearing structures, and spore arrangement are important identification aids. Light in the near ultraviolet range is required for sporulation in some fungi, especially those with dark spores. Relatively few references^{5,6} provide appreciable help in identifying cultural recoveries. Table III provides a key to the most common fungus types producing growth on widely used media, while many of these are depicted in Figure 2.

TABLE I. KEY TO SOME COMMON AIRBORNE POLLENS OF NORTH AMERICA

For experienced observers, pollen identification is a single step recognition process, and any preliminary discriminative processing is subconscious. However, for the unfamiliar, particle identity must be deduced by stepwise comparison of surface, apertures, etc. with those of similar entities. This process is systematically followed in using a key like that below. Each step requires a specific decision and the choices elected lead to subsequent decision steps and, finally, to the particle's identity. Success requires that the key treat each particle type for which it is used and that structural features are properly observed and interpreted (i.e., the choices are valid). This key is intended for particles trapped on a sticky surface and mounted in a water-based medium containing fuchsin. It is designed to treat most acknowledged pollen allergens as well as certain other types which may cause confusion. Given access to the dye, essentially all airborne pollens stain with fuchsin. Although a few spore types also "blush" in this medium, *unstainable* particles will not be found in this table.

- 1a) Single unit, generally spherical or ovoid —————→ 3
- 1b) Tetrad (inseparable group of 4 units) —————→ 2
- 1c) Polyad — roughly spherical grouping of 16 flattened units (frost-free areas) *Acacia*
- 1d) Single unit with 2 lateral (often air-filled) bladders —————→ Conifers [pines, spruces, firs, *Podocarpus*, mountain hemlock (Northwest)]

- 2a) Square tetrad, each grain 20 to 28 μm . with single irregular pore; surface prominently reticulated —————→ Broad-leaved cattail
- 2b) Tetrahedral tetrad, no obvious apertures —————→ *Luzula* (wood rush)
- 2c) Tetrahedral tetrad, grains with evident slender furrows —————→ Heath family*

- 3a) Particle without furrows or pores (= inaperturate) —————→ 4
- 3b) Particle with one or more pores only (= porate) —————→ 38
- 3c) Particle with furrows but no pores (= colpate) —————→ 33
- 3d) Particle with furrows containing central pores (colporate) —————→ 39

- 4a) Grain 70 to 80 μm . warty surface and single bladder as a fringe or collar —————→ Canadian hemlock (*Tsuga*)
- 4b) "Grains" with trilete (triradiate) scar —————→ Spores of ferns, clubmosses, other nonflowering plants
- 4c) Grain without collar or trilete scar —————→ 5

- 5a) Grain 60 to 90 μm . in diameter, surface appearing smooth — Larch, tamarack
- 5b) Grain below 50 μm . in diameter —————→ 6

- 6a) Grain with single irregular projection (exit papilla), 22-32 μm . —————→ Bald cypress (South), sequoias (West)
- 6b) Grain lacking exit papilla —————→ 7

- 7a) Grain appearing smooth-surfaced, outer layer (exine) thin and often shed as a unit in mounting. Intine thick with irregular stellate protoplast, usually 25 to 35 μm . —————→ Juniper, yew, cypress group
- 7b) Exine as definitely granular patches on surface; stellate protoplast absent, usually 42 to 46 μm . —————→ Aspen, poplar (*Populus*)
- 8a) Grain with single pore at tip of exit papilla (see 6a) —————→ Bald cypress (South), sequoias, (West)
- 8b) Grain single-pored; without papilla —————→ 9
- 8c) Grain with two or more pores; without papilla —————→ 12

- 9a) Surface reticulate; spherical or nearly so, pore more or less distinct; 27 to 38 μm . —————→ Narrow leafed cattail (*Typha angustifolia*, also bur-reed [*Sparganium*])
- 9b) Surface granular or smooth —————→ 10

- ***A refractile wall thickening surrounding a pore.

- 23a) Exine thickened at pore margin; subtriangular in polar view —————→ Hazelnut
(*Corylus*)
- 23b) Exine not thickened at pore margin —————→ 24
- 24a) Subtriangular in polar view (sub-tropical areas) —————→ Australian pine (*Casuarina*)
- 24b) Subspherical in polar view; shed in spring; accept fuchsin staining well-
—————→ Ironwood, hornbeam (*Ostrya*, *Carpinus*)
- 24c) Subspherical in polar view; shed in Summer; stain faint red-violet with fuchsin
—————→ Hemp (*Cannabis*), hops (*Humulus*)
- 25a) Pores 4 —————→ 26
- 25b) Pores 5 or more ('stephanoporate') —————→ 27
- 26a) Pore areas joined by curved ridges (arci) —————→ Alder (*Alnus*)
- 26b) Curved ridges lacking —————→ Sweet fern (also aberrant grains
of birch family and osage
orange)
- 27a) Pores 5 in equatorial plane —————→ 28
- 27b) Pores more than 5, scattered over surface ('periporate') —————→ 29
- 28a) Pores aspidate, margin joined by curved ridges —————→ Alder (*Alnus*)
- 28b) Pores aspidate, lackingarci —————→ Aberrant grains of birches,
hackberry, etc.
- 28c) Pores not aspidate, indistinct; grain surface with peanutlike convolutions;
grains pentagonal in polar view —————→ Elm (*Ulmus*)
- 29a) Pores 6 - 15, located on equator and one hemisphere; grains often flattened, angular
in polar view —————→ Walnut (*Juglans*)
- 29b) Pores distributed on both hemispheres, grains subspherical —————→ 30
- 30a) Grain less than 20 $\mu\text{m.}$; pores 9 to 11 only, usually 3-4 $\mu\text{m.}$ wide ———
—————→ Meadow rue (*Thalictrum*)
- 30b) Grain larger than 20 $\mu\text{m.}$ —————→ 31
- 31a) Pores 12 to 20 with bulging pore membranes bearing dark granules; pore diameters
variable, usually 5 $\mu\text{m.}$, grains >30 $\mu\text{m.}$ in diameter —————→ Sweet gum
(*Liquid-
ambar*)
- 31b) Grains with <12 pores or, if more, without bulging, granule-flecked pore mem-
branes —————→ 32
- 32a) Pores 6 to 11, each ca. 3 $\mu\text{m.}$ in diameter and with a small central operculum —
—————→ Plantain (*Plantago*)
- 32b) Pores 14 to over 75 —————→ Chenopod-Amaranth group
- 33a) One often-indistinct and contracted furrow present; grains elongate, 24 to 36
 $\mu\text{m.}$; wavy exine markings border the furrow (temperate areas) —————→ *Ginkgo*
- 33b) Single furrow, broad, clearcut; grains 18 to 26 $\mu\text{m.}$; surface featureless (tropical
and subtropical area) —————→ Date palm (*Phoenix*)
- 33c) Furrows 3 or more, without pores —————→ 34
- 33d) Furrows 3, each with a central pore —————→ 39
- 34a) Grains 20 $\mu\text{m.}$ or less —————→ 35
- 34b) Grains larger than 20 $\mu\text{m.}$ —————→ 36
- 35a) Surface pitted; furrows relatively short with indistinct ends —————
—————→ Sycamore (*Platanus*)
- 35b) Surface with netlike reticulations —————→ Tamarix (occasional small
willow grains are indis-
tinguishable)
- 36a) Grains tetracolpate (or rarely five-furrowed); furrows jagged, lacking sharp
margins; surface with fine-netted reticulation —————→ Ash (*Fraxinus*)
- 36b) Grain tricolpate —————→ 37

- 37a) Grains with distinct netlike reticulation becoming finer near furrows; grains prolate; furrow ends distinct, generally 24 μm . \longrightarrow Willow (*Salix*)
- 37b) Grain surface finely or coarsely granular but without definite netlike reticulation; size generally 26 μm . \longrightarrow 38
- 38a) Grains coarsely and irregularly granular, exine staining a dark reddish purple; furrows jagged, often bulging, causing recurved exine at furrow margins; grains oblate; subtriangular in polar view \longrightarrow Oak (*Quercus*)
- 38b) Grains finely granular, often forming delicate parallel striae; staining moderately intense violet; furrows with distinct margins, seldom bulging; grains prolate; subspherical in polar view \longrightarrow Maple (*Acer*)†
- 39a) Grain markedly elongate, furrows thin, with prominent central pore or short transverse furrow \longrightarrow 40
- 39b) Grains spherical or triangular, but not elongate \longrightarrow 41
- 40a) Long axis of grain <18 μm .; furrows relatively long \longrightarrow Chesnut (*Castanea*)
- 40b) Long axis of grain 20 to 40 μm .; furrows short, equator often somewhat constricted \longrightarrow Carrot family (*Umbelliferae*)
- 41a) Exine greatly thickened around apertures; grains triangular (polar view) with apertures between angles \longrightarrow Linden, basswood (*Tilia*)
- 41b) Exine thickenings absent; grains spherical or, if triangular, with furrows at the angles \longrightarrow 42
- 42a) Grain surface distinctly spiny \longrightarrow 43
- 42b) Grain surface reticulate, smooth or granular, but not spiny \longrightarrow 47
- 43a) Grain with interconnected ridges (= Lophate) bearing spines; apertures in 6-sided lacunae \longrightarrow Dandelion, chickory, and certain other composites
- 43b) Ridges lacking; entire grain surface spiny \longrightarrow 44
- 44a) Spines <2 μm . in length \longrightarrow 45
- 44b) Spines >2 μm . in length \longrightarrow Entemophilous composite type‡
- 45a) Grain <23 μm .; conical spines clearcut \longrightarrow 46
- 45b) Grain >24 μm .—spines often minute \longrightarrow Cocklebur§ (*Xanthium*)
- 46a) Apertures indistinct; may appear as pores only \longrightarrow Ragweeds¹¹ (*Ambrosia*)
- 46b) Apertures clearly evident with elongate furrows \longrightarrow *Iva*¹¹
- 47a) Grain surface with netlike reticulum \longrightarrow 52
- 47b) Grain surface smooth or granular \longrightarrow 48
- 48a) Surface coarsely granular; grains >32 μm ., spherical \longrightarrow Beech (*Fagus*)
- 48b) Surface smooth, finely granular; usually <32 μm . \longrightarrow 49
- 49a) Internal starch granules prominent; furrows long, very thin, three or four in number \longrightarrow Dock, sorrel (*Rumex*)
- 49b) Internal starch granules absent; pore width <2 \times that of enclosing furrow; furrows 3 \longrightarrow 50
- 50a) Exine distinctly thickened midway between furrows; walls with short radial columns (bacculae) \longrightarrow Sage (*Artemisia*)
- 50b) Neither interfurrow thickenings nor bacculae present \longrightarrow 51

†Of all domestic maples, box elder is most like oak in size and surface markings; absolute distinctions often are impossible even with the multiple features listed.

‡Includes asters, golden rods, ornamental composites, and some wind-pollinated types (e.g., *Baccharis*).

§Certain *Iva* and cocklebur species have pollens that are essentially typical of ragweeds. Pollen of *Iva xanthifolia* (but not *I. ciliata*) is clearly colpate.

¹¹See footnote with couplet 45.

- 51a) Exine with bandlike equatorial thickening; pores with recurved "lips" in polar view; furrows distinct; grain subspherical (frost-free areas) → Castor bean (*Ricinus*)
- 51b) Equatorial band lacking; pores without "lips"; pores bulging, sharply margined; furrow margins indistinct; grain subtriangular (southwest) → Mesquite (*Prosopis*)
- 52a) Mesh of netlike reticulation diminishes at poles and lengthens to parallel furrows (East) → *Ailanthus*
- 52b) Mesh of reticulation unchanged at poles and not elongate at furrows → 53
- 53a) Pore membranes bulging; subtriangular in polar view (warm Southwest) → Silk-tassel tree (*Garrya*)
- 53b) Pore membranes not bulging; subspherical in polar view (warm Southwest) → Olive (*Olea*)^a

TABLE II. A KEY TO COMMON AIRBORNE DARK-SPORED IMPERFECT FUNGI

- 1) With both transverse and longitudinal septa → 2
- 1) No septa or transverse only → 6
- 2) Spherical, warty, very dark brown → *Epicoccum* (6)*
- 2) Not spherical → 3
- 3) Clavate (broader at one end), one end tapering to a broad appendage → *Alternaria* (8)
- 3) Without an appendage, ellipsoid or broadly ovate → 4
- 4) Broadly ovate to subspherical, constricted in center → *Stemphylium* (7)
- 4) Not constricted → 5
- 5) With hyaline attachment appendage; three transverse septa with two middle cells divided longitudinally → *Pithomyces* (10)
- 5) Without appendage, less regularly septate → *Ulocladium* (9)
- 6) With transverse septa only → 7
- 6) With no septa → 10
- 7) Thickwalled with very thick, usually light-colored septa → *Drechslera*
Helminthosporium (12)
Corynespora
Sporidesmium
- 7) Without thick septa; septa usually dark → 8
- 8) Broadly elliptic, strongly curved, with lighter end cells → *Curvularia* (11)
- 8) Not consistently curved → 9
- 9) Very dark, constricted at septa → *Torula* (13)
Dendryphiella (15)
- 9) Not strongly constricted; with both nonseptate and septate cells present → *Cladosporium* (4)
Dendryphion (14)

^aSpecies of privet (*Ligustrum*) shed small amounts of similar pollen.

*Numbers in parentheses refer to spore illustrations in Figure 1.

- 10) Very black, nearly spherical (slightly flattened at poles) → *Nigrospora* (2)
 10) Not black or not spherical → 11
 11) Dark brown, disk shaped with light equatorial slit → *Arthrinium* (1)
 11) Not as above → 12
 12) Gold brown with dark brown warts, spherical → *Periconia* (3)
 12) Not as above → 13
 13) Pale tan (nearly colorless); broadly ovate, thin-walled, often collapsed, with one apparent flattened attachment point → *Botrytis* (5)
 13) Pale tan to golden brown; elliptical to cylindrical, with refractive scars at both ends → *Cladosporium* (4)

TABLE III. SOME TAXA FREQUENTLY RECOVERED FROM AIR WITH A CULTURE-PLATE SAMPLER

Part I. Examine plate visually; look for:

- 1) Colonies with visible, (small), discrete, sessile fruiting bodies that can be individually picked up with fine forceps → Ascomycetes (25)
 IIA
 Sphaeropsids (24)
 2
 1) Without this combination of characters →
 2) Colonies that fill the plate both vertically and laterally and have faintly grey to black "fruiting" structures on thin stalks → *Mucor* (26)
 IIB
Rhizopus
 3
 2) Without this combination of characters →
 3) Small orange-brown colonies less than 1 mm. in diameter → *Wallemia* (6)
 4
 3) Larger and/or not orange-brown →
 4) Very fragile white mycelium, with pink-purple pigment diffusing into the culture medium (colony often ring-shaped with thin center and fluffy margin) → *Fusarium* (12)
 5
 4) Without this combination of characters →
 5) Colony smooth and shiny or pastey (yeastlike) → 6
 5) Colony mycelial (cottony, felted, powdery, hairy) → 8
 6) Colony surface becoming mottled or streaked with brown or black → *Aureobasidium* (3)
 7
 6) Colony not mottled →
 7) Colony surface and reverse black → *Phialophora* (11)
 and other black yeasts
 Yeasts, bacteria
Candida
 7) Colony surface and reverse some other color →
 8) Reverse side of colony dark brown to black or mottled dark and light (ignore translucent agar) → IIC
 8) Colony reverse light or brightly colored → 9

- 9) Colony surface yellow to yellow/orange, spreading broadly, colony reverse dark orange-red → *Epicoccum* (14)
 9) Without this combination of characters → 10
 10) Colony reverse and surface white → IID
 10) Colony surface some other color (including pale tan or pinkish) → 11
 11) Colony surface some shade of green → 12
 11) Colony surface some other color → IIE
 12) Colony spreading rapidly, overgrowing the plate, with small compact masses of bright green spores → *Trichoderma* (10)
 12) Colony green but compact and often powdery to velvety with spores → IIF

Part II. Make a slide and microscopically look for:

- A) 1) Spores inside sacs within the fruit body, often 8 spores/sac (crush young fruiting bodies to see) → Ascomycetes
 e.g., *Chaetomium* (25)
 2) Spores not in sacs, often extremely abundant → Sphaeropsids
 e.g., *Phoma* (24)
- B) 1) Mycelium very broad; spore-bearing stalks thickening toward base and bearing rhizoids (rootlike structures) → *Rhizopus*
 1) Mycelium less broad, without rhizoids → *Mucor* (26)
- C) 1) Spores mostly unicellular, occasionally some with transverse septa only → 2
 1) Spores regularly multicellular → 6
 2) Spores with refractive connector areas at one or both ends, borne on spore-bearing structures in chains → *Cladosporium* (4)
 2) Without this combination of characters → 3
 3) Spores small ($< 5 \mu$), nearly colorless, borne at apex of flask-shaped cells → *Phialophora* (11)
 3) Without this combination of characters → 4
 4) Spores dark brown to black → 5
 4) Spores pale tan, tear-drop shaped, thin-walled, borne on large branching spore stalks → *Botrytis* (23)
 5) Spores nearly spherical, black → *Nigrospora* (17)
 5) Spores lozenge-shaped, dark brown → *Arthrinium* (18)
 6) Spores with transverse septa only, septa thick → *Helminthosporium*
Drechslera (15)
 6) Spores with both transverse and longitudinal septa → 7
 7) Spores solitary on small pegs, often a piece of the peg remains attached to the base of the spore → *Pithomyces* (16)
 7) Spores with a tapering or cylindrical apical projection, found in chains (but chains breaking up with mounting) → *Alternaria* (13)
- D) 1) Mycelium fragmenting into more or less cylindrical spores → Arthrospores
 e.g., *Geotrichum* (1)
 1) Without this combination of character → 2

- | | | |
|-------|---|--|
| 2) | Spores formed in branched or unbranched chains by budding; individual spores may also bud _____ | <i>Monilia</i> (2) |
| 2) | Without this combination of characters _____ | 3 |
| 3) | Spores borne in groups or chains at the apex of long tapering or flask-shaped cells _____ | <i>Cephalosporium</i>
<i>Fusarium</i> |
| 3) | Spores borne on denticles _____ | 4 |
| 4) | Apex of spore-producing cell elongating with spore production _____ | <i>Tritarachium</i> (9) |
| 4) | Apex of spore-producing cell swelling but not elongating _____ | <i>Sporothrix</i> (8) |
| E) 1) | Spores elongate-fusoid, borne in long chains on long thin cells; colony tan to pink _____ | <i>Paecilomyces</i> (7) |
| 1) | Spores ovate-subglobose or colony not tan or pink _____ | 2 |
| 2) | Colony tan or pinkish, spores ovate subglobose, borne on flask shaped cells with cylindrical apices _____ | <i>Scopulariopsis</i> (5) |
| 2) | Without this combination of characters _____ | IIF |
| F) 1) | Spore-bearing stalk with enlarged bulb at apex _____ | <i>Aspergillus</i> (21, 22) |
| 1) | Spore-bearing stalk with apical branches, without enlarged bulb _____ | <i>Penicillium</i> (19, 20) |

This key includes some of the most commonly isolated airborne fungi. During an extended sampling program other organisms will surely be recovered that are not included. Reference 5 should be consulted for help with fungi that do not fit the key. Characteristics listed are based on sporulating material grown for ~ 7 days on malt extract agar. Numbers in parentheses that follow the listed fungus types refer to drawings of Figure 2. Letters correspond to sections of Part II of the key.

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